REMARKS

Application Amendments

By the amendments presented, the specification is rewritten on Page 6 to indicate that the Figure 8 description refers back to "Figure 7" and not to a non-existent "Figure 22A". That this should be the case is clearly shown elsewhere in the specification at Page 21, lines 22-25.

Also by the amendments presented, the specification is rewritten on Page 11, lines 24-31 to delete the embedded hyperlink and to replace it with reference to an internet website instead.

Also by the amendments presented, the specification is rewritten at Page 47, line 31 to indicate that the amino acid sequence of the CPI3 peptide described on that line corresponds to SEQ ID No. 23. This is shown to be the case elsewhere in the specification at Page 8, lines 29-30.

Also by the amendments presented, Claim 1 has been rewritten in a number of places. In the first place, Claim 1 has now been recast as a "method of detecting the presence or absence of a microorganism of interest in a sample" by detecting modification of a substrate "exposed to said sample". Support for this characterization of the claimed method can be found in the original specification, for example, from Page 6, line 30 to Page 31, line 3 and at Page 37, line 6 and lines 11-13.

Further by the amendments presented, Claim 1 is further rewritten to indicate that the substrate modification results from exposure to a protein produced by any of said microorganism of interest which may be present in the sample being tested. Support for this Claim 1 amendment is found in the original specification at Page 29, at lines 19-20 and lines 30-32.

Further by the amendments presented, Claim 1 is still further rewritten to indicate that the substrate modification comprises cleaving a portion of the

peptide comprising the first colorimetric component from the substrate. Support for this Claim 1 amendment is found in the original Claim 13 of the application.

Further by the amendments presented, Claim 1 is still further rewritten to indicate that the visible color change of the method is one which is perceptible without any kind of detection equipment or enhancement equipment. Support for this Claim 1 amendment is found in the original specification at Page 7, lines 7-8.

Further by the amendments presented, Claim 1 is still further rewritten to indicate that the peptide component of the substrate has an amino acid sequence which permits the substrate to specifically and uniquely react with the protein produced by the microorganism of interest. Support for this Claim 1 amendment is found in the original specification at Page 37, lines 5-13.

Further by the amendments presented, Claim 1 is still further rewritten to indicate that the first colorimetric component of the substrate used in the claimed method comprises a reactive dye approved for use in foods, drugs, cosmetics or medical devices by the U.S. Food & Drug Administration. Support for this Claim 1 amendment is found in the original specification at Page 14, lines 16-18.

Further by the amendments presented, Claim 13 is rewritten to delete therefrom the portions of that claim which have now been incorporated into amended Claim 1.

Further by the amendments presented, non-elected Claims 16-22 have been cancelled without prejudice.

Upon entry of the claim amendments presented, Claims 1-15 remain in the application. No additional claims fee is due as a result of these claim amendments.

Invention Synopsis

The present invention as now claimed is directed to a method for detecting the presence or absence of a microorganism of interest in a sample which can be, for example, a wound surface or a body fluid or other fluid taken from a wound. Detection of the presence or absence of the microorganism of interest is

carried out by exposing a certain kind of substrate to the sample and detecting whether or not a modification of the substrate occurs.

In the first step of this method, an unmodified substrate comprising a peptide having a first colorimetric component coupled thereto is exposed to the sample under conditions such that a protein, for example an enzyme, produced by any of the microorganism of interest which might be present in the sample will modify the substrate. Then in the second method step any modification of the substrate which does occur is detected, or if no modification occurs, that absence of modification is detected.

The detectable substrate modification is one which involves cleaving of a portion of the peptide containing the first colorimetric component. It is this substrate cleaving which results in a color change that is visible to the naked eye without the use of any kind of detection equipment or enhancement equipment.

The substrate employed in the method herein comprises a peptide component having an amino acid sequence which permits the substrate to specifically and uniquely react with the protein produced by any selected specific microorganism of interest which the method is designed to detect. The first colorimetric component of the substrate comprises a reactive dye which is approved for use in foods, drugs, cosmetics or medical devices by the U.S. Food & Drug Administration.

Restriction/Election Requirements

In the instant Office Action, the previously applied restriction and election requirements have now been made FINAL. Accordingly, the previously withdrawn, non-elected Claims 16-22 are cancelled herein without prejudice. Applicants expressly preserve their right to pursue such cancelled, non-elected claims via one or more divisional applications. The species which applicants have elected are those set forth in their previous June 12, 2009 Response to the election requirement.

Formal Matters

Drawings

The drawings originally presented with this application have been objected to because the images of Figures 5 - 9, 16 and 17 are allegedly unrecognizable. In response to this drawing objection, applicants are submitting herewith 8 sheets of consolidated formal drawings which include all of the original Figures 1-17. Since some of the figures in these new drawings are in the form of color photographs, the 8 new sheets of formal drawings are accompanied by the requisite Petition Under 37 CFR 1.84. It is submitted that presentation of these new formal drawing sheets obviates the objection to the drawings set forth in the instant Office Action.

Specification Objection

In the instant Office Action, several objections to the specification have been raised. In the first place, the Examiner indicates that the specification contains improper embedded hyperlinks on Pages 11 and 27. (Applicants' attorney can find no hyperlink at Page 27, line 21 as indicated by the Examiner.) By the amendments presented herein, the "http://" prefix has been removed from the website listed at Page 11, line 27. Such an amendment obviates this basis for objection to the specification.

In the instant Office Action, objection has also been raised to the lack of a sequence identifier for the amino acid sequence listed at the end of Page 47. The appropriate sequence identifier has now been added to the listing of the CPI3 peptide given on Page 47. This addition obviates this basis for the specification objection.

In the instant Office Action, objection has also been made to the reference on Page 6 to a "Figure 22A" when describing Figure 8. The reference to "Figure 22A" has now been changed to "Figure 7". This amendment thus obviates this basis for the specification objection.

Rejection Under 35 U.S.C. §112, Second Paragraph

In the instant Office Action Claims 1-15 have been rejected under 35 U.S.C. §112, Second Paragraph, for allegedly being indefinite in the use of the terms "sample" and "modification". The Examiner contends that it is unclear "what activity in the sample" is being detected and that it is unclear what type of "modification" is being detected by the claimed method. Such a rejection is respectfully traversed in light of the claim amendments made herein.

It is submitted that it is quite clear from the instant specification that a "sample" being tested in accordance with the method now claimed herein refers to any material, article or location wherein a microorganism of interest might be present. Applicants' specification at Page 33, lines 4-16 and in original Claim 12 provides an extensive list of the kind of materials, articles and locations which can comprise the sample being tested. In light of this disclosure, it is submitted that the skilled artisan would have no difficulty in determining when and how a "sample" is tested in accordance with the claimed method.

It is further submitted that it is now quite clear from the amended Claim 1 provided herein that the modification of the substrate which occurs in the context of the method claimed herein refers to the cleaving of a portion of the specific peptide which forms an essential component of the substrate used and to which the first colorimetric component is coupled. Given this express reference in the claims to the mechanism of modification, it is submitted that the skilled artisan would have no difficulty in determining if, how and when such a modification occurs in the context of the presently claimed method.

In light of the foregoing considerations, it is apparent that the amended Claim 1 provided herein uses terminology which clearly defines how and in what context the method claimed therein is to be practiced. Accordingly, it is submitted that the amended Claim 1 and the claims dependent therefrom are in complete compliance with the definiteness requirements of 35 U.S.C. §112, Second Paragraph.

Art Rejections

Claims 1-5, 7-12 and 15 have been rejected under 35 U.S.C. §102(a) as allegedly being directly anticipated by Sanders et al (WO 03/063693, hereinafter referred to as "Sanders"). The Examiner contends that Sanders discloses all of the elements (e.g., substrate formed from peptide and coupled colorimetric component which is cleaved when contacted with a sample to produce a visible color change) of the rejected claims. Such a rejection is respectfully traversed as it would apply to the claims as amended herein.

Sanders discloses methods for detecting the presence or absence of wound pathogens by contacting a sample such as wound fluid with a substrate and detecting a modification of that substrate caused by enzymes produced and/or secreted by wound bacteria. The Sanders substrates can include peptides with described amino acid sequences. Such substrates in Sanders can be labeled with detectable markers such a fluorescent dyes which produce detectable fluorescent changes when the substrates are modified. Sanders also discloses that chromogenic dyes such a p-nitrophenol which produce visible color changes can be used as substrate markers.

It is submitted that the Sanders reference fails to directly anticipate applicants' Claims 1-5, 7-12 and 15 as amended herein. There is no disclosure in Sanders of applicants' use as colorimetric markers of dyes which are approved by the U.S. Food & Drug Administration for food, drug, cosmetic or medical device use. The one chromogenic dye mentioned in Sanders is para-nitrophenol which is toxic and could never be used in methods which involve contact of this material with, or incorporation of this material into, the human body.

It should further be noted that Sanders fails to disclose the use in detection methods of colorimetric components which produce a visible color change that can be detected without the use of detection or enhancement equipment. While para-nitrophenol can generate a pale yellow color which is within the visible range of the electromagnetic spectrum, its color can be masked by body fluids such as blood or wound fluid. Accordingly, as indicated in Sanders at Page 16, lines 18-25, even though p-nitrophenol can produce a

visible color change, its use in Sanders to indicate progress of the enzyme – substrate interaction is still monitored by measuring absorbance at 415 nm using a spectrophotometer

Given the foregoing considerations, it is submitted that Sanders fails to disclose all of the elements of applicants' detection method as currently described in the claims rejected over this reference. Continued rejection of amended Claims 1-5, 7-12 and 15 under 35 U.S.C. §102(a) over the Sanders reference would therefore be improper.

Claims 1, 3, 5 and 12 have also been rejected under 35 U.S.C. §102(b) as allegedly being directly anticipated by Asai et al. (*Clinica Chimica Acta*, 1984, 144, pp. 163-171), hereinafter "Asai"). The Examiner again contends that Asai discloses all of the elements of applicants' invention as set forth in the rejected claims. Such a rejection is also respectfully traversed as it would apply to these rejected claims as amended herein.

The Asai reference discloses a colorimetric assay for antithrombin III in plasma. Such an assay uses PS-915, a peptide substrate which liberates 3-carboxy-4-hydroxyaniline as part of a colorimetric method. Antithrombin III is not a protein which is produced by a microorganism in a sample as is used in applicants' claimed method. Rather it is a host protease produced in the liver and found in plasma. As with the Sanders reference, the carboxy hydroxy aniline which is liberated from the PS-915 substrate in Asai is not a reactive dye which is approved for food, drug, cosmetic or medical device use by the FDA. CHA is, in fact, not disclosed in Asai as being a dye at all, but rather in the Asai assay produces a blue color only when complexed with another added reagent which is alkaline-pentacyanoammine ferroate. And finally, as in Sanders, the Asai method uses a spectrophotometer to measure color intensity, and accordingly the Asai method is not one like applicants' which must be carried out without the use of color intensity detection equipment.

In short, the Asai reference fails to disclose a number of essential elements of applicants' detection method as now described in the rejected

claims. Continued rejection of applicants' amended Claims 1-3, 5, and 12 under 35 U.S.C. §102(b) over this Asai reference would therefore be improper.

Claims 1-3, 5, 8 and 11-15 have also been rejected under 35 U.S.C. §102(b) as allegedly being directly anticipated by Kulisek et al. (*Anal. Biochem.*, 1989, 177, pp. 78-84), hereinafter "Kulisek"). The Examiner again contends that Kulisek discloses all of the elements of the rejected claims. Such a rejection is also respectfully traversed as it would apply to the rejected claims as amended herein.

Kulisek discloses a chromogenic assay for the detection of plasmin. In this assay plasmin is generated by a plasminogen activator immobilized on nitrocellulose. The assay uses a p-nitroanilide synthetic peptide substrate which is cleaved by plasmin to release p-nitoaniline which is subsequently coupled to a naphthylethylene diamine to produce a diazoammonium salt of intense red color.

As with the Sanders and Asai references, the Kulisek reference fails to disclose a microorganism detection method which utilizes peptide-bound reactive dyes which are approved for food, drug, cosmetic or medical device use by the USFDA. The p-nitroaniline released in the Kuliesek method is toxic and would never be sued in a medical grade device. Furthermore, p-nitroaniline substrates such as used in Kulisek would be set off by a number of host proteases making them poor substrates to detect infection *in vivo*.

As with the other references used in the rejections herein under 35 U.S.C. §102, the Kulisek reference, as noted, fails to disclose all of the essential elements of the method of the rejected claims as now written. Again therefore, continued rejection of these claims as directly anticipated by Kulisek would be improper.

Finally, Claims 1 and 6 have been rejected under 35 U.S.C. §103(a) as allegedly being unpatentably obvious over Kulisek in view of Graham et al (*Ann Appl. Biol.*, 1995, 127, pp 163-173, hereinafter "Graham"). The Examiner contends that Graham discloses that p-nitroaniline dye released in the Kulisek

method can be detected either as color reduction or elimination of color, thereby rendering the rejected claims obvious. Such a rejection is also respectfully traversed as it would apply to the amended claims herein.

Graham discloses a "visual" assay technique developed to identify plants expressing Cowpea protease trypsin inhibitor. Since trypsin acts on an α-N-benzoyl-DL-arginine-p-nitroanilide (BAPNA) substrate to produce p-nitroaniline, trypsin inhibition is identified by detecting a reduction or elimination of yellow color development which occurs as the hydrolysis of BAPNA is allowed to proceed.

It is apparent that Graham does not rectify any of the deficiencies of Kulisek *vis à vis* a teaching or suggestion of applicants' claimed method. Like Kulisek, the Graham method utilizes the toxic p-nitroaniline as the color indicator. And Graham thus adds nothing to, and in fact reinforces, the Kulisek failure to show methods such as applicants' which must use FDA approved reactive dyes. Graham furthermore clearly teaches away from the methods of applicants' Claims 1 and 6 by measuring the loss or reduction in p-nitroaniline color intensity using a spectrophotometer.

Given the foregoing considerations, it is submitted that the reference combination of Kulisek in view of Graham fails to suggest the methods of either of applicants' amended Claims 1 or 6. Continued rejection of these two claims under 35 U.S.C. §103 over this reference matrix would therefore be improper.

Double Patenting Rejection

Claims 1, 8, 11 and 15 have been provisionally rejected on the grounds of obviousness double patenting over Claims 1, 16, 18, 20, 35, 37, 39, 53, and 57 of a copending application having U.S. Serial No. 10/502,882. Since neither the present nor the copending application have yet been allowed, applicants will address the obviousness double patenting issue once allowable subject matter has been identified in both applications.

Conclusions

Applicants have made an earnest effort to place their application in proper form and to distinguish their claimed invention from the applied prior art. WHEREFORE, reconsideration of this application, entry of the claim amendments presented herein, acceptance of the new sheets of formal drawings, withdrawal of the drawings and specification objections and the claim rejections under 35 U.S.C. §§112, 102 and 103, and allowance of Claims 1-15 as amended, are all respectfully requested.

Any comments or questions concerning this application can be directed to the undersigned at the telephone number given below.

Respectfully submitted,

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